bundles to the membrane of gut microvilli [16], where they are thought to drive membrane towards the tips of protrusions. Although it is not yet clear whether myosin I homologues play a similar role in the fly, Myo31DF and Myo61F are found localized to the brush borders of fly hindgut epithelial cells soon after the gut has taken on its characteristic sinistral twist [11]. In addition, over-expression of the ERM-protein Moesin, another key regulator of microvilli structure, randomizes the direction of Myo31DF and Myo61F dependent L–R morphogenesis [10], suggesting a link between L–R symmetry-breaking and microvilli. As Myo31DF and Myo61F are both expected to move towards the barbed ends of actin filaments, the functional antagonism between these motors is likely to be mediated by differences in their cargo. The conserved IQ domains of myosin I may also play a role since they are essential for symmetry breaking [9], and target a mouse myosin I to brush borders [17]. In a search for relevant cargo, Speder *et al.* [9] identified beta-catenin bound to the tail domain of Myo31DF. Intriguingly, beta-catenin also binds Inversin, the only protein capable of reversing L–R determination in the mouse [18–20], where it may be linked to cadherin-mediated adhesion or Wnt signalling. These data point to a possible link between these processes in the mouse and fly. Clearly, more has to be done before we understand the functions of Myo31DF and Myo61F in L–R symmetry-breaking. But, given the excitement generated by these findings, we will soon learn whether the functions of myosin I motors in other bilateral animals mirror those of Myo31DF and Myo61F in the fly.

References

1. Wood, W.B. (1998). Handed asymmetry in nematodes. Semin. Cell Dev. Biol. *9*, 53–60.
2. Wandelt, J., and Nagy, L.M. (2004). Left-right asymmetry: more than one way to coil a shell. Curr. Biol. *14*, R654–R656.
3. Hirokawa, N., Tanaka, Y., Okada, Y., and Takeda, S. (2006). Nodal flow and the generation of left-right asymmetry. Cell *125*, 33–45.
4. Nonaka, S., Tanaka, Y., Okada, Y., Takeda, S., Harada, A., Kanai, Y., Kido, M., and Hirokawa, N. (1998). Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. Cell *95*, 829–837.
5. Okada, Y., Nonaka, S., Tanaka, Y., Saijoh, Y., Hamada, H., and Hirokawa, N. (1999). Abnormal nodal flow precedes situs inversus in iv and inv mice. Mol. Cell *4*, 459–468.
6. Nonaka, S., Shiratori, H., Saijoh, Y., and Hamada, H. (2002). Determination of left-right patterning of the mouse embryo by artificial nodal flow. Nature *418*, 96–99.
7. Levin, M. (2005). Left-right asymmetry in embryonic development: a comprehensive review. Mech. Dev. *122*, 3–25.
8. Shibazaki, Y., Shimizu, M., and Kuroda, R. (2004). Body handedness is directed by genetically determined cytoskeletal dynamics in the early embryo. Curr. Biol. *14*, 1462–1467.
9. Speder, P., Adam, G., and Noselli, S. (2006). Type ID unconventional myosin controls left-right asymmetry in Drosophila. Nature *440*, 803–807.
10. Hozumi, S., Maeda, R., Taniguchi, K., Kanai, M., Shirakabe, S., Sasamura, T., Speder, P., Noselli, S., Aigaki, T., Murakami, R., *et al.* (2006). An unconventional myosin in Drosophila reverses the default handedness in visceral organs. Nature *440*, 798–802.
11. Morgan, N.S., Heintzelman, M.B., and Mooseker, M.S. (1995). Characterization of myosin-IA and myosin-IB, two unconventional myosins associated with the Drosophila brush border cytoskeleton. Dev. Biol. *172*, 51–71.
12. Hayashi, T., and Murakami, R. (2001). Left-right asymmetry in Drosophila melanogaster gut development. Dev. Growth Differ. *43*, 239–246.
13. Brown, N.A., and Wolpert, L. (1990). The development of handedness in left/right asymmetry. Development *109*, 1–9.
14. Hayashi, M., Aono, H., Ishihara, J., Oshima, S., Yamamoto, H., Nakazato, Y., and Kobayashi, S. (2005). Left-right asymmetry in the alimentary canal of the Drosophila embryo. Dev. Growth Differ. *47*, 457–460.
15. Ali, M.Y., Uemura, S., Adachi, K., Itoh, H., Kinosita, K., Jr., and Ishiwata, S. (2002). Myosin V is a left-handed spiral motor on the right-handed actin helix. Nat. Struct. Biol. *9*, 464–467.
16. Tyska, M.J., Mackey, A.T., Huang, J.D., Copeland, N.G., Jenkins, N.A., and Mooseker, M.S. (2005). Myosin-1a is critical for normal brush border structure and composition. Mol. Biol. Cell *16*, 2443–2457.
17. Cyr, J.L., Dumont, R.A., and Gillespie, P.G. (2002). Myosin-1c interacts with hair-cell receptors through its calmodulin-binding IQ domains. J. Neurosci. *22*, 2487–2495.
18. Simons, M., Gloy, J., Ganner, A., Bullerkotte, A., Bashkurov, M., Kronig, C., Schermer, B., Benzing, T., Cabello, O.A., Jenny, A., *et al.* (2005). Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. Nat. Genet. *37*, 537–543.
19. Nurnberger, J., Bacallao, R.L., and Phillips, C.L. (2002). Inversin forms a complex with catenins and N-cadherin in polarized epithelial cells. Mol. Biol. Cell *13*, 3096–3106.
20. Morgan, D., Goodship, J., Essner, J.J., Vogan, K.J., Turnpenny, L., Yost, H.J., Tabin, C.J., and Strachan, T. (2002). The left-right determinant inversin has highly conserved ankyrin repeat and IQ domains and interacts with calmodulin. Hum. Genet. *110*, 377–384.

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T-Cell Memory: The Importance of Chemokine-Mediated Cell Attraction

A recent study demonstrates the involvement of certain chemokines in immune response initiation and CD8$^+$ T-cell memory formation. These seminal findings broaden our understanding of the role of chemokines in adaptive immune processes.

Bernhard Moser

Chemokines represent a class of pro-inflammatory cytokines that have the ability to attract and activate leukocytes. Our current knowledge about chemokines and adhesion molecules underscores the strict relationship between leukocyte localization and leukocyte function [1,2]. In the case of T- and B-cell responses, three key events are regulated by chemokines, namely pathogen contact and processing in the tissue, immune response initiation in the draining lymph node, and pathogen neutralization by newly generated, pathogen-specific effector cells. Recent work by Ron Germain and colleagues [3] now demonstrates that chemokines

facilitate the interactions between CD8$^+$ T cells, CD4$^+$ T cells and dendritic cells in the lymph node and promote CD8$^+$ T-cell memory formation. Here I shall discuss the importance of the new findings using as an example the primary infection of human skin by molluscum contagiosum (MC) virus, a poxvirus inducing strong CD8$^+$ T-cell responses.

Infection with viruses, such as MC virus, induces local innate responses, including expression of stress factors and proinflammatory cytokines. In response, resident macrophages, dendritic cells and tissue cells secrete a variety of inflammatory chemokines leading to the first step in the immune response against MC virus (Figure 1). Naïve T cells, the antigen-inexperienced precursors that give rise to effector T cells, do not respond to inflammatory chemokines and are largely excluded from inflamed peripheral tissues. The transition of naïve T cells into effector/memory T cells occurs in secondary lymphoid tissues, such as axillary lymph nodes draining the portion of skin infected with MC virus (Figure 1). Lymph nodes are highly specialized organs in which blood-derived, naïve T cells come into contact with antigen-presenting cells presenting peptides from tissue-derived pathogens in the so-called T zones. Lymph nodes also harbor naïve, mature B cells in the follicular compartments (B zones) where pathogen-selective antibody responses are initiated.

Since naïve T cells do not get access to MC virus at the site of infection, the virus needs to reach the draining lymph node, either via drainage of particulate viral antigens in fluid phase or via cellular transport of the whole virus. Interstitial dendritic cells make up the prototype antigen-transport/presenting system [4], but lymphocytes may also fulfill this function under special circumstances [5]. Dendritic cells within tissues endocytose pathogenic components, such as debris of MC virus-infected epidermal keratinocytes, and process these into major histocompatibility

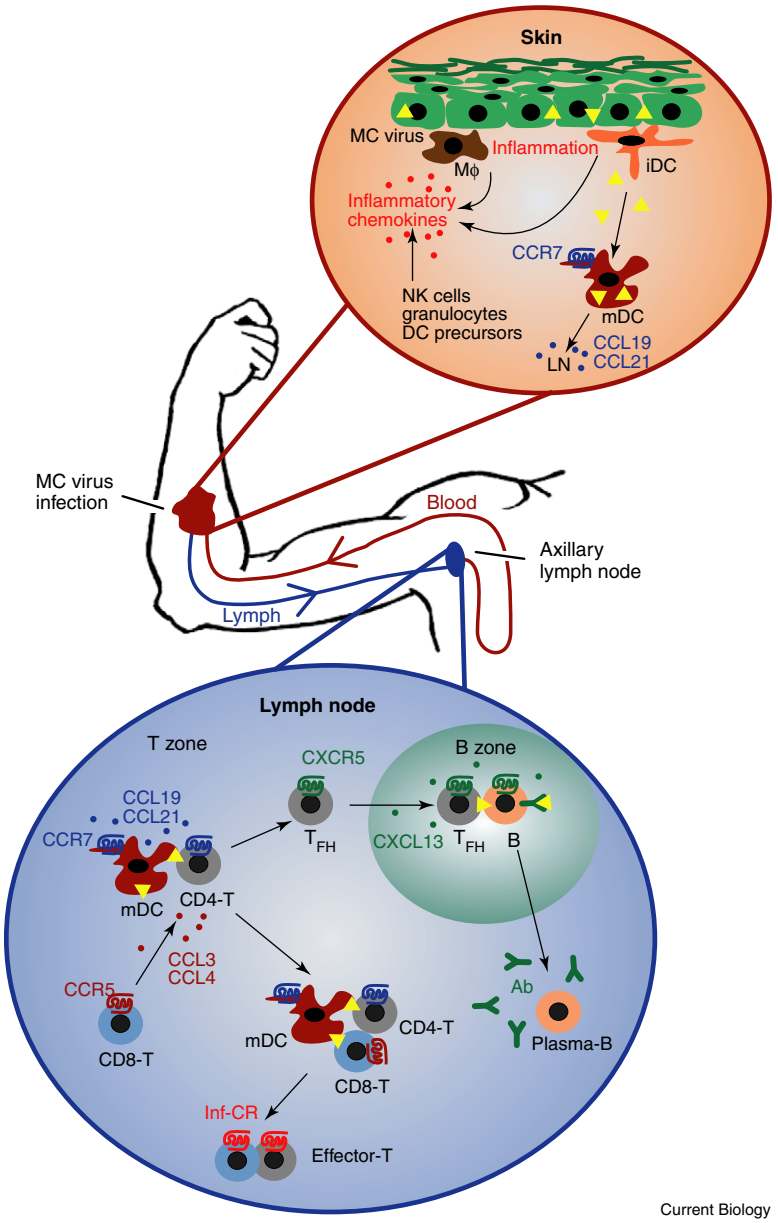

Figure 1. Leukocyte relocation in the course of MC virus infection.

MC virus infection of keratinocytes within human epidermis initiates sequential leukocyte relocation steps involving: lymph node homing of antigen-presenting dendritic cells and naïve T cells; T cell–dendritic cell and T cell–B cell interactions in the T zone and B zone of the axillary lymph node; and relocation of newly generated, MC virus-specific effector T cells to the MC virus infection site. MC, molluscum contagiosum; DC, dendritic cell; iDC/mDC, immature/mature DC; Mϕ, macrophage; CD4-T/CD8-T, CD4$^+$ T cell/CD8$^+$ T cell; Effector-T, effector T cell ; T_{FH}, follicular B helper T cell; B, B cell; Plasma-B, plasma cell; Ab, antibody; Inf-CR, receptors for inflammatory chemokines.

complex (MHC)–peptide complexes that are displayed on the dendritic cell surface [6]. Alternatively, dendritic cells can also serve as transport vehicles of unprocessed antigen. At the site of infection and inflammation, dendritic cells undergo a crucial transformation, termed 'maturation', which entails two key processes: stabilization of antigen-presentation and T-cell co-stimulation features; and substitution of their inflammatory migration program with a 'lymph node homing' program [4]. The migratory changes involve the *de novo* expression of the chemokine receptor CCR7, which allows mature dendritic cells to respond to the chemokines CCL19 and CCL21 expressed on lymphatic vessels

and high endothelial venules that feed lymph nodes and dendritic cells within the T zone of lymph nodes. Naïve T cells in peripheral blood also express CCR7 and respond to these T-zone chemokines. Collectively, CCR7 and its two selective chemokines CCL19 and CCL21 are an integral part of the lymph node homing program, which contributes to the second step in the course of adaptive immune responses, namely, the co-localization of antigen-presenting dendritic cells and naïve T cells (Figure 1) [1,7].

It is interesting to note that immature dendritic cells present in healthy peripheral tissues also appear to depend on this lymph node homing receptor for induction of tolerance against self-antigens [8]. Similarly, some effector/memory T cells in peripheral tissues may also use CCR7 for lymph node entry, although their precise role in intranodal immune processes remains to be determined [9,10]. Finally, a subset of memory T cells in peripheral blood expresses this receptor and uses it for continuous access to lymph nodes and spleen [7].

Lymph nodes are structurally divided into T-cell and B-cell compartments. The T-cell compartment is characterized by dynamic interactions between antigen-presenting dendritic cells and naïve T cells, which may lead to T cell priming and effector/memory T-cell generation [11]. Localisation of naïve $CD8^+$ T cells to the T zone may not fully depend on CCR7 and CCL19/CCL21, although contact with MHC class I–peptide-presenting dendritic cells and $CD4^+$ T cells in the T zone is mandatory for $CD8^+$ T-cell activation [12]. In the recent study, Ronald Germain and his group [3] now show that $CD4^+$ T cells produce the chemokines CCL3 and CCL4 during their engagement with antigen-presenting dendritic cells. Interestingly enough, these chemokines contribute to the recruitment of naïve $CD8^+$ T cells to the site of $CD4^+$ T cell–dendritic cell interactions within the T zone of lymph nodes, thus enabling the assembly of all three cellular players. Neutralization of CCL3 and CCL4 reduced the extent of co-localization of these three cell types and, surprisingly, had an unfavorable effect on the development of a long-lasting $CD8^+$ T-cell memory.

This remarkable finding merits further discussion. $CD4^+$ T cells are known to secrete chemokines (and cytokines) upon T-cell receptor triggering. In most instances, these chemokines belong to the broad category of inflammatory chemokines, which attract effector cells to sites of inflammation [1]. However, a subset of $CD4^+$ T cells synthesizes the homeostatic chemokine CXCL13, which orchestrates the co-localization of $CD4^+$ T helper cells and B cells in the B-cell compartment during the initiation of B-cell responses [13]. In brief, $CD4^+$ T-cell priming in the T zone of lymph nodes results in *de novo* expression of activation and memory markers as well as CXCR5, the receptor that is selective for CXCL13 and is uniformally expressed in mature, naïve B cells. This special $CD4^+$ T-cell subset, termed follicular B helper T cells (T_{FH} cells), becomes engaged in B-cell differentiation and plasma cell generation (Figure 1) [13]. In analogy to the recently described effects of T-zone-produced CCL3 and CCL4 [3], T_{FH} cell-derived CXCL13 may contribute to sequential contacts between B cells and T_{FH} cells within the B-cell compartment. At present, there is no evidence to support a direct effect of CXCL13 on B-cell differentiation, suggesting that the primary role of this chemokine is the control of co-localization between T cells and B cells. The situation might be different for the T-zone chemokines CCL19 and CCL21, which, in addition to their role in the co-localization of T cells and dendritic cells (see above), may also directly affect T helper cell differentiation [14,15]. Whether or not the CCL3 and CCL4 chemokines produced by $CD4^+$ T cells fulfill similar functions in $CD8^+$ T-cell differentiation requires further examination.

In the scheme outlined by Germain and colleagues [3], naïve $CD8^+$ T cells must express CCR5, the only receptor for CCL4 and one of two receptors for CCL3 (Figure 1). It is important to note that naïve and resting $CD8^+$ T cells do not express CCR5 or any other type of receptor for inflammatory chemokines. The stimuli that induce CCR5 expression in naïve $CD8^+$ T cells have not yet been identified but may be part of the highly complex process of leukocyte extravasation [16]. On a final note, the observed effect of CCL3/CCL4 on $CD8^+$ T-cell memory formation provokes additional questions, including those concerning the spatial and temporal parameters dictating the fate of $CD8^+$ T cells in the course of effector *versus* memory cell differentiation.

In the MC virus model, interactions between MC virus peptide-presenting dendritic cells and naïve, MC virus-specific T cells in the T zone of lymph nodes results in the generation of MC virus-specific effector T cells. $CD4^+$ T cells enhance dendritic cell maturation through CD40 engagement and, in return, fully mature dendritic cells provide soluble and cell-associated factors for the differentiation of $CD4^+$ T cells as well as $CD8^+$ T cells. As discussed above, newly primed $CD4^+$ T (T_{FH}) cells are also required for mounting MC virus-specific B-cell responses. At the effector stage of adaptive immune responses MC virus-selective effector T cells are no longer retained in the draining lymph node [17]. Instead, they display a migration program that enables the third and final step in our model of skin infection (Figure 1). This step depends fully on the expression of those chemokine receptors that target the MC virus-specific effector T cells to the infected skin tissue. As a final remark, this third step of cellular co-localization does not account for the regulation of homing of long-lasting memory T cells. In fact, in order to avoid unwarranted tissue damage, the chemokine system(s) controlling memory T-cell traffic in healthy tissue should not attract short-lived effector T cells [18,19]. The study by Germain and his group [3] underscores a remarkable relationship between early events

in naïve CD8$^+$ T-cell activation and CD8$^+$ T-cell memory formation and suggests that these early events may also affect the migratory properties in long-lasting memory CD8$^+$ T cells.

References

1. Moser, B., Wolf, M., Walz, A., and Loetscher, P. (2004). Chemokines: multiple levels of leukocyte migration control. Trends Immunol. *25*, 75–84.
2. Sallusto, F., and Mackay, C.R. (2004). Chemoattractants and their receptors in homeostasis and inflammation. Curr. Opin. Immunol. *16*, 724–731.
3. Castellino, F., Huang, A.Y., Altan-Bonnet, G., Stoll, S., Scheinecker, C., and Germain, R.N. (2006). Chemokines enhance immunity by guiding naive CD8+ T cells to sites of CD4+ T cell-dendritic cell interaction. Nature *440*, 890–895.
4. Banchereau, J., and Steinman, R.M. (1998). Dendritic cells and the control of immunity. Nature *392*, 245–252.
5. Brandes, M., Willimann, K., and Moser, B. (2005). Professional antigen-presentation function by human gammadelta T cells. Science *309*, 264–268.
6. Villadangos, J.A., and Heath, W.R. (2005). Life cycle, migration and antigen presenting functions of spleen and lymph node dendritic cells: limitations of the Langerhans cells paradigm. Semin. Immunol. *17*, 262–272.
7. Sallusto, F., Geginat, J., and Lanzavecchia, A. (2004). Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu. Rev. Immunol. *22*, 745–763.
8. Ohl, L., Mohaupt, M., Czeloth, N., Hintzen, G., Kiafard, Z., Zwirner, J., *et al.* (2004). CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. Immunity *21*, 279–288.
9. Bromley, S.K., Thomas, S.Y., and Luster, A.D. (2005). Chemokine receptor CCR7 guides T cell exit from peripheral tissues and entry into afferent lymphatics. Nat. Immunol. *6*, 895–901.
10. Debes, G.F., Arnold, C.N., Young, A.J., Krautwald, S., Lipp, M., Hay, J.B., *et al.* (2005). Chemokine receptor CCR7 required for T lymphocyte exit from peripheral tissues. Nat. Immunol. *6*, 889–894.
11. Von Andrian, U.H., and Mempel, T.R. (2003). Homing and cellular traffic in lymph nodes. Nat. Rev. Immunol. *3*, 867–878.
12. Junt, T., Scandella, E., Forster, R., Krebs, P., Krautwald, S., Lipp, M., *et al.* (2004). Impact of CCR7 on priming and distribution of antiviral effector and memory CTL. J. Immunol. *173*, 6684–6693.
13. Vinuesa, C.G., Tangye, S.G., Moser, B., and Mackay, C.R. (2005). Follicular B helper T cells in antibody responses and autoimmunity. Nat. Rev. Immunol. *5*, 853–865.
14. Flanagan, K., Moroziewicz, D., Kwak, H., Horig, H., and Kaufman, H.L. (2004). The lymphoid chemokine CCL21 costimulates naive T cell expansion and Th1 polarization of non-regulatory CD4+ T cells. Cell. Immunol. *231*, 75–84.
15. Marsland, B.J., Battig, P., Bauer, M., Ruedl, C., Lassing, U., Beerli, R.R., *et al.* (2005). CCL19 and CCL21 induce a potent proinflammatory differentiation program in licensed dendritic cells. Immunity *22*, 493–505.
16. Randolph, G.J., Beaulieu, S., Lebecque, S., Steinman, R.M., and Muller, W.A. (1998). Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. Science *282*, 480–483.
17. Cyster, J.G. (2005). Chemokines, sphingosine-1-phosphate, and cell migration in secondary lymphoid organs. Annu. Rev. Immunol. *23*, 127–159.
18. Schaerli, P., Ebert, L., Willimann, K., Blaser, A., Roos, R.S., Loetscher, P., *et al.* (2004). A skin-selective homing mechanism for human immune surveillance T cells. J. Exp. Med. *199*, 1265–1275.
19. Ebert, L.M., Meuter, S., and Moser, B. (2006). Homing and function of human skin gammadelta T cells and NK cells: relevance for tumor surveillance. J. Immunol. *176*, 4331–4336.

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Sex Determination: Time for Meiosis? The Gonad Decides

Germ cell sex determination is directed by the gonad and is characterized by a difference in the timing of entry into meiosis. Recent data show that retinoic acid signalling is responsible for the induction of germ cell meiosis in the developing ovary. In the fetal testis, this process is inhibited by a retinoic acid metabolizing enzyme.

Amanda Swain

The role of germ cells is to ensure the transmission of genetic information from one generation to the next. To achieve this, the organism must protect and nurture these cells through meiosis so that they develop into haploid gametes, either sperm or eggs. The germ cell lineage in the mouse appears midway through gastrulation as a small cluster of primordial germ cells at the base of the allantois [1]. During development, these cells migrate through the hindgut and arrive at the gonad as it forms. This process has no sexual bias; however, after their arrival in the gonad, germ cells follow a sex specific fate. In the female, germ cells enter the first meiotic prophase and become oogonia at around 13.5 days *post coitum* (dpc). In the male, germ cells arrest in the G1/G0 stage of the cell cycle and become so-called T-prospermatogonia. After birth, they will resume mitosis and then enter meiosis a week later. Therefore, the first sexual difference in behaviour between male and female germ cells is their timing of onset into meiosis. It has been known for some time, from studies mainly from Anne McLaren's group, that this difference was independent of the sex chromosome constitution of germ cells but was dependent on their gonadal environment [2–5]. Now, two exciting papers by Koubova *et al.* [6] and Bowles *et al.* [7] show that retinoic acid (RA) signalling is part of the switch that determines whether germ cells will develop as oocytes or as prospermatogonia.

The identification of two genes with particular gonadal expression patterns pointed towards the involvement of RA signalling in the control of the initiation of germ cell meiosis during embryogenesis. The RA-responsive gene *Stra8* was first identified in a screen for genes induced by RA in embryonal carcinoma cells. Analysis of its expression pattern in the developing gonad showed that it was specific to germ cells of female (XX) embryos at a stage and pattern that preceded the onset of meiosis in the ovary by a day [8]. This suggested that *Stra8* was an early marker of meiotic initiation. At 13.5 dpc, the gene *Cyp26b1*, which encodes an enzyme that metabolizes RA to an inactive form, was found to be expressed exclusively in the somatic cells of the male (XY) gonad [9]. These expression patterns suggested that RA might be responsible for